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Interplay between dental pulp tissue osteoprotegerin and TNF-α levels with micromorphological changes in the teeth of patients with chronic pulpitis

Interakcija između nivoa osteoprotegerina i TNF-α u dentalnoj pulpi sa mikromorfološkim promenama zuba kod pacijenata sa hroničnim pulpitisom

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Abstract

Background/Aim. Chronic pulpitis (CP) is an inflammatory dental pulp disorder associated with various pathophysiological mechanisms in its origin. The aim of the study was to evaluate the changes in the pulp tissue of osteoprotegerin (OPG) and tumor necrosis factor (TNF)-α and establish their relationship with the histological changes of pulp tissue, as well as with the micromorphological changes, occurring in the mineralized tissue. **Methods.** The study examined the dental pulp of 41 patients with CP and 12 healthy individuals. The group of the diseased subjects was subdivided based on the presence of communication of the pulp with the oral cavity, i.e., open (n = 22) or closed (*clausa*) (n = 19) CP. **Results.** The levels of TNF-α were statistically significantly increased, while OPG levels were decreased in the pulp of patients with CP, compared to the control group. TNF-α levels were almost the same in the pulp of patients with closed CP and the control group, while values were significantly increased in those with open CP compared to control. Histopathological analysis showed a significant increase in the number of mononuclear inflammatory cells in the diseased pulp. Scanning electron microscopy showed distinct changes, which correlate with internal resorption. **Conclusion.** The results indicate a much greater and intensified process of root resorption in patients with closed CP, which is unassociated with dental pulp OPG and TNF-α level changes.

Key words:
cytokines; dental pulp; histological techniques; microscopy, electron, scanning; osteoprotegerin; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. Hronični pulpitis (HP) je inflamacijski poremećaj zubne pulpe, u čijoj osnovi se nalaze različiti patofiziološki mehanizmi. Cilj rada bio je da se procene promene osteoprotegerina (OPG) i faktora nekroze tumora (*tumor necrosis factor*-TNF)-α u tkivu pulpe i ustanovi njihova povezanost sa histološkim promenama tkiva pulpe, kao i sa mikromorfološkim promenama koje se javljaju u mineralizovanom tkivu. **Metode.** Studijom je ispitana zubna pulpa 41 pacijenta sa HP i zubna pulpa 12 zdravih osoba. Grupa obolelih osoba podeljena je na osnovu prisustva komunikacije pulpe sa usnom dupljom, odnosno otvorenog (n = 22) ili zatvorenog (*clausa*) (n = 19) HP. **Rezultati.** Nivoi TNF-α bili su statistički značajno povišeni, dok je nivo OPG bio snižen u pulpi pacijenata sa HP, u poređenju sa kontrolnom grupom. Nivoi TNF-α bili su skoro jednaki u pulpi pacijenata sa zatvorenim HP i kontrolnoj grupi, a značajno povišeni kod pacijenata sa otvorenim HP u odnosu na kontrolnu grupu. Histopatološkom analizom uočeno je značajno povećanje broja mononuklearnih inflamacijskih ćelija u bolesnoj pulpi. Skenirajućom elektronskom mikroskopijom pokazane su jasne promene, koje su bile u korelaciji sa unutrašnjom resorpcijom. **Zaključak.** Rezultati ukazuju na znatno veći i intenzivirani proces resorpcije korena kod pacijenata sa zatvorenim HP, koji nije povezan sa promenama nivoa OPG i TNF-α u dentalnoj pulpi.

Ključne reči:
citokini; zub, pulpa; histološke tehnike; mikroskopija, elektronska, skenirajuća; osteoprotegerin; faktor nekroze tumora alfa.

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Introduction

Although teeth are located in a small area of the human body, the oral cavity, their unique structure and function, as well as their interaction with the rest of the body, make them a very complex structure to study. Teeth are comprised of outer mineralized tissues (dentin, enamel, cementum) that surround and protect the soft tissue within (dental pulp) [1]. The structure of the dental pulp, apart from the connective tissue, includes numerous cells, among which dental pulp stem cells have a vital role in dentine regeneration, multilineage differentiation, etc. [2]. The secretion of cytokines and chemokines by the cells in this milieu, as well as the influence of extra pulpal stimuli, are responsible for the dynamics of the processes occurring within the pulp. The cytokine level disbalance, especially during inflammatory stimulation, is responsible for the disturbances in both mineralized and soft tissue, accompanied by subjective symptoms. Dental pulp disorders can be caused by numerous factors influencing exposed pulpo-dentinal tissue and most frequently involve a progression of dental caries caused by different bacterial species [3].

Inflammatory and immune responses within the dental pulp are elicited in reaction to microorganisms, encompassing both anaerobic and aerobic bacterial species, along with their products that permeate dentinal tubules [1, 2]. The pathological process of internal root resorption leads to dentine loss and the potential invasion of the cementum due to activities originating within pulp [1]. Pulpitis, an inflammatory condition affecting dental pulp, is characterized by accumulating inflammatory cells and different mediators to specific regions [3]. Depending on the type of pulp changes and whether it occurs in an open or a closed pulp cavity, a distinction can be made between ulcerative and hyperplastic pulpitis. Chronic open pulpitis is characterized by vasodilation, infiltration of mononuclear inflammatory cells, and the occurrence of exudation, as well as cellular infiltration by neutrophil leukocytes [4]. Microscopic analysis of the tissue affected by pulpitis reveals granulation tissue comprised of new and immature capillary networks rich in inflammatory cells [5]. The infiltration of the pulp that occurs at the beginning of this process is comprised mainly of mononuclear cells (MNCs) such as lymphocytes and monocytes [1, 6]. We can make a difference between the two forms of chronic closed pulpitis – pulpitis *clausa* alternative *seu* parenchymatosa and granulomatosa interna. Closed pulpitis is identified by the infiltration of small round cells and concurrent varying degenerative changes. The pathohistological analysis of internal granuloma reveals the existence of granulation tissue surrounded with odontoclasts [5]. The inflamed area encompasses almost all types of leukocytes but mainly includes neutrophils and various forms of MNCs [7]. Cells in the infiltrate produce locally large amounts of inflammatory mediators, as well as other cytokines and chemokines [3]. The extent of inflammation can represent a diagnostic issue, which further influences the therapeutic option.

Although numerous studies reveal the mechanism underlying pulpitis, the exact nature of these biological processes is still unclear [3, 5, 7]. Modern-day laboratories, molecular biology and genetic-based, are equipped with sophisticated equipment adequate for quantitative and qualitative determination of cytokines in body fluids or tissue cell cultures obtained from different sources. Cytokines or biological response modulators include various proteins that affect inflammation, immunity, and hematopoiesis. Altered levels of cytokines have been found in inflamed pulp tissue (interleukins (IL), such as IL-8 and IL-2); however, their exact function is not completely understood [5]. The assessment of biomarkers can help improve the prediction or treatment of inflammatory pulp disease, and their investigation might thus prove to be of great value.

Osteoprotegerin (OPG) and receptor activator of nuclear factor κβ (RANK) ligand (RANKL), together with the associated RANK receptor, are proteins that share a great homology with tumor necrosis factor (TNF) receptor superfamily and are involved in the bone metabolism and osteoclastic cell function [5]. OPG is expressed by odontoblasts, ameloblasts, and dental pulp cells [8] and is involved in the maintenance of bone mineral matrix homeostasis [9]. Namely, cells expressing RANKL, such as osteoblasts, previously believed to be the only ones responsible for osteoclastic activity, and periodontal ligament fibroblasts could modulate and/or initiate root resorption [7]. During the process of root resorption induced by orthodontic forces, there is a significant shift in OPG and RANKL levels, as well as in the level of different cytokines in the pulp [5, 10]. The OPG/RANKL/RANK system could be referred to as a crucial connection in the interaction between bone, vascular, and immune cells, which is not fully understood [5]. These data suggest an association between cytokines and mineralized tissue destruction, potentially originating from the cells present in the dental pulp.

The balance that exists between pro- and anti-inflammatory cytokines predetermines the response of the body toward antigen stimulation in both acute and chronic inflammatory states [11]. The stimulation of inflammatory response causes the production of various cytokines, among which TNF-α plays an important role [12]. This cytokine induces an increase in RANKL levels in the cell, which further engages in the process of mineralized tissue destruction [5]. Moreover, TNF-α is known to be associated with the development of periodontal inflammation and the destruction of periodontal tissue [13]. These facts suggest that numerous stimuli are capable of inducing the destruction of mineralized tissue; however, their exact process remains unclear. The extent of pulp inflammation could be correlated with the progression of mineralized tissue destruction, and its estimation could help determine the course of treatment. However, up to now, there are no objective, quantitative, or clinically practical methods to assess process [4]. Likewise, there are no studies correlating changes in TNF-α levels with the inflammatory cells present in the pulp tissue or with changes in mineralized tissue ultrastructure.

The aim of the study was to determine dental pulp OPG and TNF-α level changes occurring in patients with chronic pulpitis (CP) (opened and closed) and compare the results with the ones obtained from healthy subjects. Furthermore, histopathological analysis (HPA) of the soft pulp tissue

obtained from the same patients would be performed in order to corroborate the biochemical findings. Additionally, this study encompassed the examination of the microstructural changes in the mineral tissue of teeth during the asymptomatic internal root resorption.

Methods

Study population

The study included a total of 53 patients from the Department of Dentistry. Before the commencement of the study, the study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Priština/Kosovska Mitrovica, Serbia (decision No. 05-83, from March 26, 2013). The study was conducted according to the standards given in the Declaration of Helsinki (revised in 2008). Each subject signed an informed consent form given by the lead researcher and was informed about the study details. Patients with CP (n = 41) were initially diagnosed based on the anamnestic data and auxiliary diagnostic methods (radiological findings). They were further subdivided into two groups based on the presence of communication of the pulp and the oral cavity, i.e., groups with open CP (n = 22) or closed (*clausa*) CP (n = 19). The pulp in the control group (n = 12) was obtained from the subjects with healthy teeth extirpated for prosthetic reasons.

Tissue homogenization and sample preparation

Dental pulp extraction was done under locoregional anesthesia with 2% lidocaine (lidocaine-chloride ampules, obtained from Hemofarm, Belgrade, Serbia) after the application of appropriate procedures to the teeth, gingiva, and mucous membrane in order to create an aseptic working environment (Elastic dental dam, Dental World, Italy). Pulp chamber trepanation was performed using a round dental burr while the cavity was prepared, and pulp extirpation was done with an instrument of appropriate size [3, 4]. Immediately after the extraction, healthy teeth were disinfected with 70% ethanol, and the enamel surface was crack-opened with a hammer. The pulpal tissue was removed from the exposed pulpal cavity using straight forceps. The extirpated content was randomly placed in a petri dish, and afterward, one-half of the mixed sample was placed in a sterile tube and snap-frozen (kept at -80 °C) prior to homogenization. The other randomly selected part of the tissue was immersed in a fixation medium for light microscopy. The process of homogenization [10% weight by volume (w/v)] was done using a Teflon® pestle in an ice-cold phosphate buffer (pH 7.4). The clear supernatant, used for determining OPG and TNF-α, was obtained by homogenate centrifugation at 4,000 × g for 15 min at 4 °C.

Determination of OPG and TNF-α concentrations

The obtained tissue homogenate supernatants were used for determining OPG and TNF-α concentrations in the pulp. The TNF-α enzyme-linked immunosorbent assay (ELISA) kit used in this study was obtained from R&D systems (Inc, Minneapolis, USA) with a standard curve range from 62.5 to 4,000 pg/mL. The OPG ELISA kit was obtained from R&D systems (Inc, Minneapolis, USA) with a standard curve range from 0.5 to 5.5 pg/mL. The obtained data are expressed as pg/mL.

Light microscopy tissue analysis

Extracted pulp tissue was fixated for 24 hr in a fixation medium [Zamboni fixative – phosphate buffered picric acid-formaldehyde fixative], after which the samples were washed with Millonig's buffer and then prefixed with 2% osmium-tetroxide. Dehydration was performed by a series of alcohol solutions of ascending concentrations (starting from 50% up to 100%), followed by propylene oxide. Prepared samples were left overnight in an epon resin mixture (Epon 820, DDSA, MNA, DMP30 as an accelerator) and were then molded and cut into semithin sections. Staining was performed using a basic fuchsin dissolved in ethanol and methylene blue dissolved in sodium tetraborate. Stained samples were examined under a polarization microscope (Olympus BX43, Olympus Corporation, Tokyo, Japan) equipped with a camera. Furthermore, the appearance of inflammatory cells and changes was examined on 5–10 randomly selected high-power fields (x40) [14], based solely on the shape of the nucleus, which enabled us to distinguish between polymorphonuclear cells and MNCs [15].

Scanning electron microscope analysis of the extracted teeth

In the case where internal root resorption of the teeth was in an advanced stage (n = 6), the teeth were extracted, washed, and left in sterile saline at 4 °C until the analysis. Furthermore, the teeth from the control group extracted due to prosthetic reasons were all analyzed by scanning electron microscopy (SEM). All samples were analyzed by a single examiner. Occlusal surfaces (2-3 mm thick) of the crown were cut circularly with the thinnest diamond fissure bur. The roots were cut with a separation disc longitudinally, thus enabling the separation of the root into the oral and vestibular half. Any superficial debris was removed by a subsequent wash in distilled water and dried with compressed air. Using separation pliers, the occlusal surfaces were separated and then separated longitudinally following the already prepared cuts. Each half was placed in a carrier and covered with gold under a vacuum. The examination on SEM was done using the JEOL-JSM-5300 microscope (JEOL, Tokyo, Japan).

Statistical analysis

The obtained data are given as mean values ± standard deviation, obtained from several different measurements. Initially, the differences in OPG and TNF-α concentrations between the two groups (control and experimental) were estimated using Student's *t*-test for two independent samples, while in the second case, when the experimental group was subdivided into two groups (open and closed CP), the differ-

ences between the three groups were compared using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test (GraphPad Prism, 8.0, San Diego, CA, USA). Probability values (p) equal to or less than 0.05 were taken to be statistically significant.

Results

The results of the present study revealed that OPG levels in healthy pulp tissue were statistically significantly higher ($p < 0.001$) than in the pulp of patients with CP. In the control group, pulpal tissue OPG values were around 50 pg/mL, while in the group of CP, values of OPG were around 10 pg/mL, i.e., some 5-fold decrease (Figure 1A). In the subsequent analysis of pulp OPG levels of the control subjects and those with closed CP and open CP, it was revealed that OPG was most significantly decreased in open CP patients, compared to both control and closed CP patients (Figure 1B).

TNF-α concentrations in the dental pulp tissue of the subjects with CP were statistically significantly ($p < 0.001$) higher than in the tissues obtained from the control group subjects. In the control group, pulpal tissue TNF-α values were below 5 pg/mL, while in the group of CP values of TNF-α were above 50 pg/mL, i.e., some 10-fold increase (Figure 2A). Analysis of the differences between TNF-α

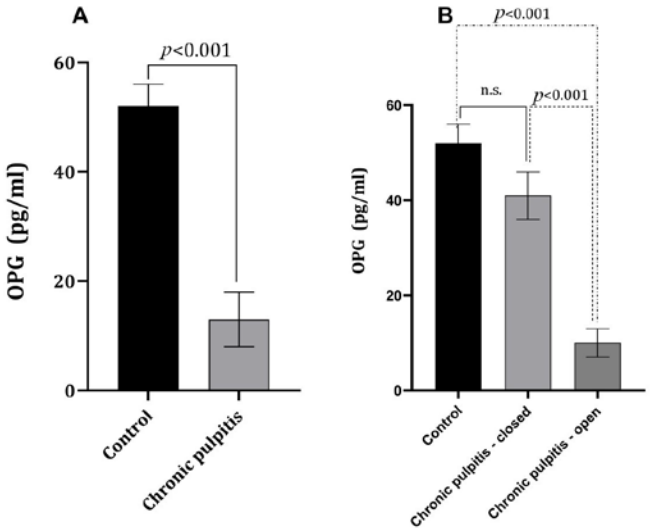

Fig. 1 – A) Comparison between the concentration of osteoprotegerin (OPG) in the dental pulp of subjects with chronic pulpitis (CP) and from the control group; B) OPG in the dental pulp of subjects with open CP, closed CP, and those from the control group. n.s. – no statistical difference; $p \leq 0.05$ was considered statistically significant [Student's *t*-test (A); ANOVA and Tukey's tests (B)].

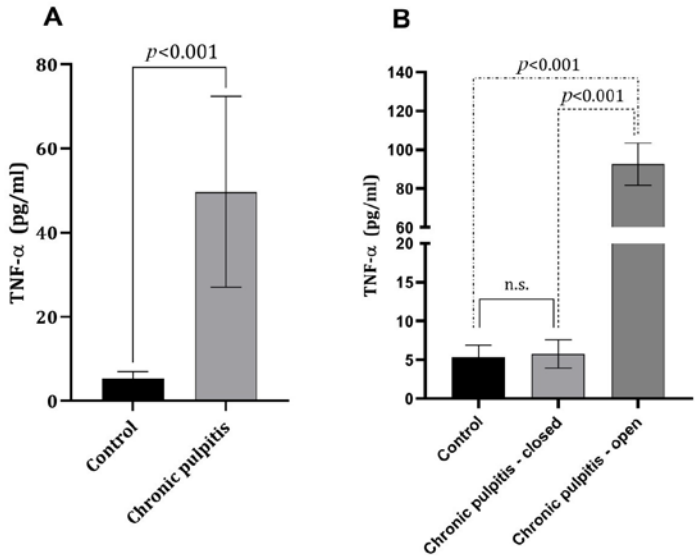

Fig. 2 – A) Comparison between the concentration of tumor necrosis factor (TNF)-α in the dental pulp of subjects with chronic pulpitis (CP) and from the control group; B) TNF-α in the dental pulp of subjects with open CP, closed CP, and those from the control group. n.s. – no statistical difference; $p \leq 0.05$ was considered statistically significant [Student's *t*-test (A); ANOVA and Tukey's tests (B)].

concentrations in the dental pulp of the control subjects and those with closed CP and open CP revealed a significant difference among the three groups ($p < 0.001$). Further analysis revealed that the levels of TNF-α in the dental pulp of the subjects with open CP were statistically significantly higher than the levels of TNF-α in the subjects belonging to either the control group or the group with closed CP. When the levels of TNF-α in the dental pulp of the subjects from the control group and the group with closed CP were compared, no statistically significant differences were found ($p > 0.05$). This means that the levels of TNF-α in the control group or the group with closed CP were around 5 pg/mL, while in the open CP, the levels were around 100 pg/mL (Figure 2B).

Analysis of the dental pulp obtained from the control group of subjects revealed a normal histoarchitecture with blood vessels, nerve fibers, and pulp cells (Figure 3A). Examination of the dental pulp with granulomatous formations that occurred during CP revealed the presence of massive necrotic fields, inflamed pulp tissue with scattered inflammatory cells, poor collagen fiber deposition, and blood vessels (sinusoids) filled with red blood cells, as well as a mass of bacteria and occasional multinuclear cell, odontoclast (Figures 3B and 3C). The number of inflammatory cells in the control group and the group of patients with closed CP was almost identical. On the other hand, the number of cells in the group of subjects with opened CP was significantly higher than in the other two groups (Figure 3D).

SEM of the teeth obtained from the subjects with healthy teeth extirpated due to prosthetic reasons showed an undamaged enamel surface, while the pulpo-dentine wall appeared regular with evenly distributed dentine canals (Figures 4A and 4B). The teeth obtained from the subjects with internal resorption revealed cracks in the enamel on the occlusal surface. The examined pulpo-dentine wall is characterized by both normal and irregular dentine structures (Figure 4C). Irregular canal distribution (Figure 4D), Howship's lacunae, and activated odontoclasts, which possess filamentous parts (filopodia) spread to the periphery (Figures 4E and 4F), sometimes up to the edge of the cracks and firmly attached to the dentine surface are seen during the SEM analysis.

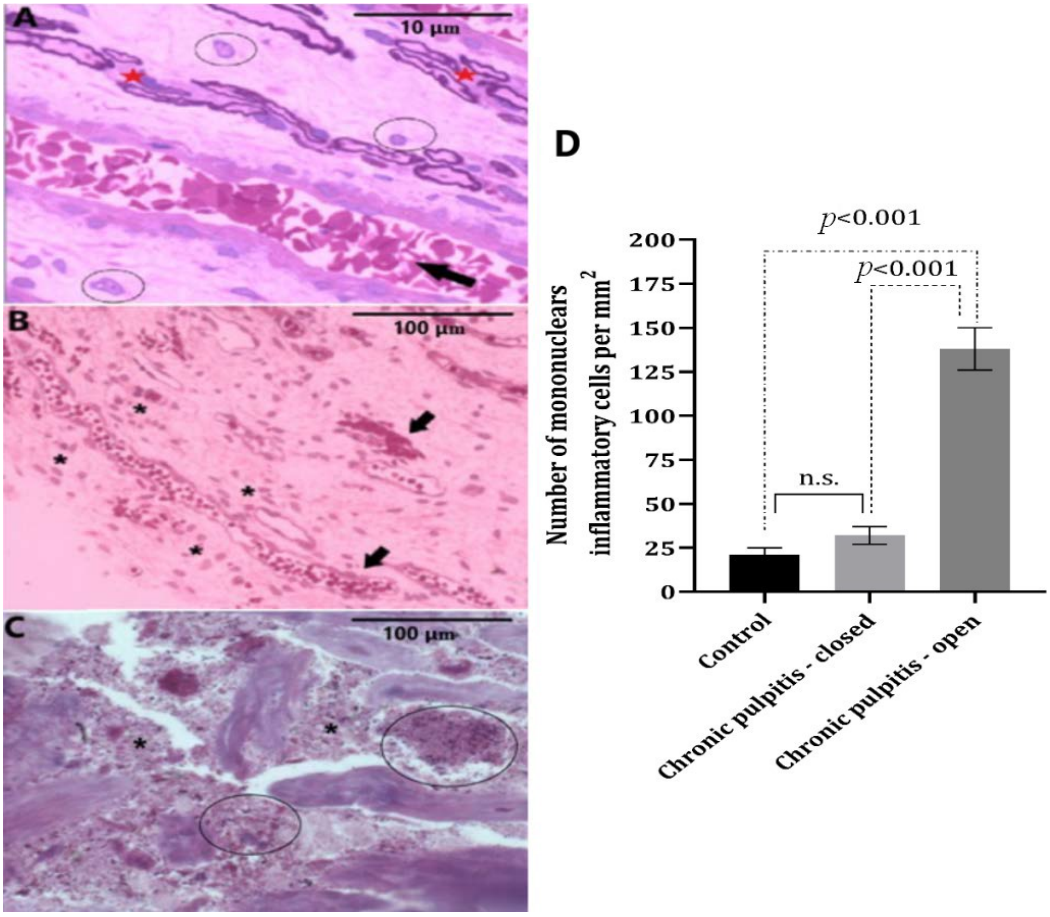

Fig. 3 – A) Histopathological appearance of the healthy dental pulp and dental pulp from patients with chronic pulpitis, normal dental pulp with red blood cells (arrow), nerve fibers (star), and dental pulp cells (circled) (×40; Fuscin staining); B) Dental pulp with hyperemia (arrow) and numerous inflammatory cells (asterisk) (×20; Fuscin staining); C) Dental pulp with necrotic field (asterisk) and bacterial infiltration (circled) (×20; Fuscin staining); D) Number of inflammatory cells in the dental pulp of subjects with chronic open pulpitis, chronic closed pulpitis, and those from the control group.
n.s. – no statistical difference; $p \leq 0.05$ was considered statistically significant (ANOVA and Tukey's tests).

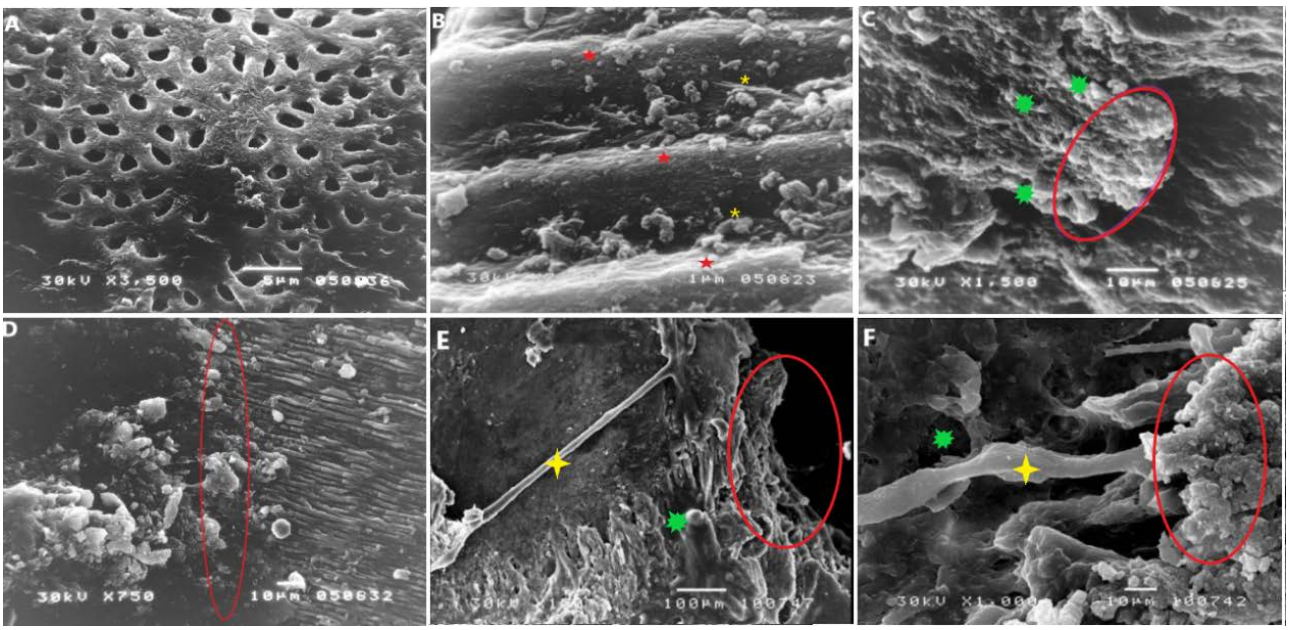

Fig. 4 – Images of healthy teeth (A and B) and teeth from patients with internal resorption (C–F) obtained by scanning electron microscopic analysis: A) regular dentine with transversely cut dental tubules and clear intertubular and peritubular dentine; B) longitudinal cut through a healthy tooth, peritubular (red star) and intratubular (yellow asterisk) dentine; C) irregular dentine structure (circled) with lacunes (multipoint star); D) the border between regular and irregular dentine (circled); E) filamentous part (four point star) of the odontoclast spreading to the surface of dentine (circled) and with formed lacunes (multipoint star); F) odontoclast filopodia (four point star) firmly attached to the damaged dentine surface (circled) with some surrounding lacunes (multipoint star).

Discussion

The destruction of the root *via* the process of internal resorption is a form of a chronic asymptomatic inflammatory disorder discovered by accident during radiography. It could be recognized as a radiolucency along the dentine surface. This inflammatory process is believed to be exclusively associated with the activity of osteoclasts. However, up-to-date studies have pointed out the role of different cells and cytokines during root resorption [4, 7]. Apart from osteoclast, fibroblasts are known to play an important role in this process, and it was proven that pulp tissue fibroblasts obtained from teeth of patients affected with root resorption in *in vitro* conditions produce significant amounts of inflammatory cytokines, e.g., TNF-α, when stimulated by the release of substance P [16]. Moreover, inflammatory cells (polymorphonuclear and mononuclear) invading dental pulp and some resident cells (fibroblasts and macrophages) could initiate and further promote internal root resorption [17]. The significant production of this cytokine is observed in patients with symptomatic pulpitis rather than in patients without symptoms [18]. This seems to be in accordance with a significantly increased number of inflammatory MNCs in the pulpal tissue of these patients (Figure 3D). Previous reports suggest that the cells comprising the inflammatory infiltrate are mainly macrophages and B cells, followed by different subsets of T cells to a much lesser extent [14]. The herein obtained data suggest that there is an increase of TNF-α in the pulp tissue of patients with CP of different origin, which could be produced by different cells observed to infiltrate the dental pulp, as well as by the resident ones. In the case of closed CP, the upper dental pulp tissue is altered and is mainly comprised of granulation tissue [19]. The removal of the dental pulp (pulpectomy), i.e., the removal of the granulation tissue and the blood supply to the process, represents one of the standard approaches in the treatment of internal root resorption [20], which was performed in patients where this process was not in an advanced stage.

The damage on the occlusal surfaces almost specifically stems from the traumatic occlusion, which further enables the invasion of the pulp and dentine with bacteria, all leading to internal resorption [3]. In some cases, during the analysis of the extracted teeth, enamel cracks on the occlusion surface were noted in the teeth analyzed by SEM. HPA of the corresponding dental pulp tissue revealed a mass of invading bacteria and inflammatory cells, mainly consisting of MNCs. These MNCs are most probably resident macrophages and/or attracted monocytes, now transformed into macrophages or belong to lymphocytes. Bacteria causing pulpitis, the ones believed to be initial invaders of the pulp, come from *Lactobacillus, Prevotella, Pseudoramibacter, Olsenella, Streptococcus,* and *Stenotrophomonas genera* [21]. Some studies suggest that without bacterial infection, the process of internal resorption would be self-limiting since the bacteria are the ones enhancing the process [22]. One of the first cells that recognize bacterial products is odontoblasts, through pattern recognition receptor toll-like receptor (TLR)-2, causing a further immune response involving cell activation, proliferation, and cytokine secretion [23]. The association between TNF-α and TLR-2 further supports the role

of TNF-α signaling in alveolar bone loss after infection with *Porphyromonas gingivalis* [24]. One of the major cell populations producing TNF-α, as well as IL-1β, is macrophages [25], the most numerous cells detected in patients with opened CP.

During the process of chronic inflammation, a cytokines could affect the dynamics of both mineral and soft tissue through the activation or inhibition of the effector cells. Namely, OPG is responsible for maintaining the symbiosis between bone resorption formation [9]. The role of OPG is the inhibition of RANK-RANKL interactions and their binding to nuclear factor (NF)-kB, thus it is essential in suppressing osteoclastogenesis and bone resorption [26]. We found that OPG levels in the healthy pulp were significantly higher than in the pulp of patients with pulpitis suggesting that the teeth bone homeostasis is maintained. This is clearly an indicator of decreased teeth mineral matrix observed both during the initial teeth examination (radiolucency) and during SEM. In some animal studies, an antibody mimicking OPG action (denosumab) was found to prevent rat root resorption when applied locally [27], further confirming the role of this molecule in root resorption.

Secreted TNF-α could also activate osteoclastic resorption [28], and, together with IL-1 lead to the activation and differentiation of osteoclasts, and induction of prostaglandin (e.g. PGE2) production from fibroblasts and osteoblasts [29]. In the present study, levels of TNF-α in the dental pulp of healthy teeth and in those with closed CP were significantly lower than the levels in the subjects with open CP, which suggests that the invasion of bacteria provoked the processes of TNF-α production. Furthermore, the produced TNF-α could originate from different inflammatory (macrophages) and residual (fibroblasts) cells, stimulated by bacterial antigens, which were both detected in the soft pulpal tissue. Apart from TNF-α, previous studies on inflamed dental pulp showed that inflammatory cells and stimulated pulpal fibroblasts strongly express and secrete IL-1β and IL-8 [30], which agrees with an increase of TNF-α and IL-1β in the same patients reported on previous occasions [4]. In addition, as mentioned, the greatest number of cells of inflammatory infiltrate is macrophages [14] and this overlaps with the findings of the present study. Interestingly, contemporary studies showed that the presence of bacterial endotoxins provokes less TNF-α production than the protein derived from dental pulp cells [31].

In this study, in the teeth analyzed using SEM and obtained mainly from the subjects with closed CP, where the extraction was indicated and performed, the resorption and evident dentine damage were always present. The active osteoclastic cells are evident in the teeth analyzed under SEM, where these cells spread with numerous filamentous parts all the way to the periphery. These findings are not fully in agreement with the increase in pulpal tissue OPG and TNF-α levels nor with the potential of these molecules to induce osteoclastic resorption as suggested elsewhere [9, 28], even in very low concentrations in the case of TNF-α [32]. Previous findings revealed an increase in pulpal tissue IL-1β [13], which should have caused, together with a local increase in TNF-α, the differentiation of osteoclasts [29]. These findings might be potentially explained by the different, non-inflammation-related, closed CP, found after certain teeth trauma or its exposure to heat [33].

The present study has a few shortcomings and weaknesses that include only descriptive SEM analysis and some morphometric analysis of the mineralized tissue ultrastructure, which might reveal additional characteristics of internal resorption that should be further performed. Moreover, further analysis of the soft tissue samples using some immunohistochemical analysis (specific molecules associated with different cell types) should reveal which cells might be the source of the cytokine production. Finally, a broader panel of cytokines, pro- and anti-inflammatory ones, could be analyzed to better understand their interactions and impact on soft and mineralized tissue function. We should also emphasize the strength of this study which lies in the multidisciplinary, biochemical, pathological, and electron microscopic approach to studying changes in the teeth under chronic inflammation.

Conclusion

The results of the present study revealed that the dental pulp tissue obtained from the subjects with open CP has significantly higher levels of TNF-α than the one obtained from the control group or the group of subjects with closed CP. Furthermore, OPG levels were found to be statistically significantly higher in the healthy dental pulp than in the one obtained from the patients with CP. The results point to the fact that OPG level changes reversely follow the changes in TNF-α levels within the dental pulp tissue. The detailed HPA of the inflamed dental pulp and the SEM, micromorphological investigation of the extracted teeth further confirmed and partially overlapped with the biochemical findings. These data suggest a much greater and intensified process of root resorption in the here studied patients with closed CP, which is based on the results unassociated with the OPG and TNF-α concentrations.

Conflict of interest

The authors declare no conflict of interest.

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